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(54) Title: RECOMBINANT CYTOMEGALOVIRUS VACCINE

(57) Abstract

The present invention provides a non-defective adenovirus recombinant expression system for the expression of an immunogenic fragment of the HCMV gB subunit, said recombinant HCMV-expressing adenovirus being useful as a vaccine.

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RECOMBINANT CYTOMEGALOVIRUS VACCINE

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Field of the Invention

The present invention refers generally to a recombinant human cytomegalovirus vaccine, and more specifically to a subunit vaccine containing fragments of a HCMV major glycoprotein complex subunit gB gene.

Background of the Invention

Cytomegalovirus (CMV) is one of a group of highly host specific herpes viruses that produce unique large cells bearing intranuclear inclusions. The envelope of the human cytomegalovirus (HCMV) is characterized by a major glycoprotein complex recently termed gB or gCI, which was previously referred to as gA. HCMV causes cytomegalic inclusion disease and has been associated with a syndrome resembling infectious mononucleosis in adults. It also induces complications in immunocompromised individuals.

CMV infection *in utero* is an important cause of central nervous system damage in newborns. Although the virus is widely distributed in the population, about 40% of women enter pregnancy without antibodies and thus are susceptible to infection. About 1% of these women undergo primary infection *in utero*. Classical cytomegalic inclusion disease is rare; however, a proportion of the infected infants, including those who were symptom-free, are subsequently found to be mentally retarded.

Preliminary estimates based on surveys of approximately 4,000 newborns from several geographical areas indicate that the virus causes significant damage of the central nervous system leading to mental deficiency in at least 10%, and perhaps as high as 25%, of infected infants. Assuming that about 1% of newborn infants per year excrete CMV and that about one fourth of those develop mental deficiency, in the United States this means approximately 10,000 brain-damaged children born per year. This is a formidable number, particularly in view of the ability of these children to survive [J. Infect. Dis., 123 (5):555 (1971)].

HCMV in humans has also been observed to cause serious complications and infections in the course of organ transplantations, especially with kidney and liver transplants.

Several HCMV vaccines have been developed or are in the process of development. Vaccines based on live attenuated strains of HCMV have been described. [See, e.g., S. A. Plotkin et al, Lancet, 1:528-30 (1984); S. A. Plotkin et al, J. Infect. Dis., 134:470-75 (1976); S. A. Plotkin et al, "Prevention of Cytomegalovirus Disease by Towne Strain Live Attenuated Vaccine", in Birth Defects, Original Article Series, 20(1):271-287 (1984); J. P. Glazer et al, Ann. Intern. Med., 91:676-83 (1979); and U. S. Patent 3,959,466.] A proposed HCMV vaccine using a recombinant vaccinia virus expressing HCMV glycoprotein B has also been described. [See, e.g., Cranage, M. P. et al, EMBO J., 5:3057-3063 (1986).] However, vaccinia models for vaccine delivery are believed to cause local reactions. Additionally, vaccinia vaccines are considered possible causes of encephalitis.

Adenoviruses have been developed previously as efficient heterologous gene expression vectors. For example, an adenovirus vector has been employed to express herpes simplex virus glycoprotein gB [D. C. Johnson et al, Virol., 164:1-14 (1988)]; human immunodeficiency virus type 1 envelope protein [R. L. Dewar et al, J. Virol., 63:129-136 (1988)]; and hepatitis B surface antigen [A. R. Davis et al, Proc. Natl. Acad. Sci., U.S.A., 82:7560-7564 (1985); J. E. Morin et al, Proc. Natl. Acad. Sci., U.S.A., 84:4626-4630 (1987)]. Adenoviruses have also been found to be non-toxic as vaccine components in humans [See, e.g., E. T. Takajuji et al, J. Infect. Dis., 140:48-53 (1970); P. B. Collis et al, J. Inf. Dis., 128:74-750 (1973); and R. B. Couch et al, Am. Rev. Respir. Dis., 88:394-403 (1963)].

There remains a need in the art for additional vaccines capable of preventing CMV infection by generating neutralizing antibody and cellular responses to CMV in the human immune system.

Summary of the Invention

In one aspect, the present invention provides a non-defective recombinant adenovirus containing a fragment of a gB subunit of the HCMV free from association with any additional human proteinaceous material. In this recombinant adenovirus, the HCMV subunit is under the control of regulatory sequences capable of expressing the HCMV gB subunit fragment *in vitro* and *in vivo*.

Another aspect of the present invention is a vaccine composition comprising a non-defective recombinant adenovirus, as described above.

In a further aspect, the invention provides a method of vaccinating a human against HCMV comprising administering to the patient the recombinant adenovirus

containing the subunit gene encoding a gB protein fragment in a vaccine composition. The inventors have found that this method of presenting these HCMV gene fragments to a vaccinate is particularly capable of eliciting an immune response.

In still a further aspect the invention provides an adenovirus-produced gB subunit fragment, which fragment may also form vaccine compositions to protect humans against HCMV. Currently, the preferred fragment comprises about amino acids 1 to about 303 of the gB protein SEQ ID NO:2, gB_{1-303}.

Other aspects and advantages of the present invention are described further in the following detailed description of preferred embodiments of the present invention.

Brief Description of the Drawings

Fig. 1A illustrates diagrammatically the cloning of the gB gene into the early region 3 (E3) transcription unit of Ad5. Represented are the 3.1kb fragment containing the gB gene by the open box; the adenovirus sequences extending from 59.5 to 100 mu (except for the deletion of the 78.5 to 84.7 mu length) by the filled portion of the circle: the large BamHI fragment of the pBR322 by the thin line of the circle. In the figure, the restriction enzymes are identified as follows: X is XbaI, B is BamHI.

Fig. 1B illustrates diagrammatically the construction of the recombinant adenovirus virus Ad5/gB, containing the gB gene of the Towne strain of HCMV described in Example 1. This figure shows the 59.5 mu to 76 mu region where homologous recombination occurs (as indicated by the crossed lines) between wild type Ad5 viral sequence and the adenovirus sequences present on the pAd5 plasmid containing the gB gene. The plaque

purified recombinant virus retains the cloning XbaI sites and the direction of transcription of the gB gene from the E3 promoter is indicated by the bent arrow. Restriction enzymes are as identified above.

Detailed Description of the Invention

The present invention provides novel immunogenic components for HCMV which comprise an adenovirus expression system capable of expressing a selected HCMV subunit gene fragment *in vivo*. Alternatively the selected subunit fragment for use in an immunogenic composition, such as a vaccine, may be expressed in, and isolated from, the recombinant adenovirus expression system.

As provided by the present invention, any adenovirus strain capable of replicating in mammalian cells *in vitro* may be used to construct an expression vector for the selected HCMV subunit. However, a preferred expression system involves a non-defective adenovirus strain, including, but not limited to, adenovirus type 5. Alternatively, other desirable adenovirus strains may be employed which are capable of being orally administered, for use in expressing the CMV subunit *in vivo*. Such strains useful for *in vivo* production of the subunit in addition to adenovirus-5 strains include adenovirus type 4, 7, and 21 strains. [See, e.g., Takajuji et al, cited above]. Appropriate strains of adenovirus, including those identified above and those employed in the examples below are publicly available from sources such as the American Type Culture Collection, Rockville, Maryland.

Similarly, a number of strains of isolated human CMV may be employed from which a desired gB subunit is derived. For example, the Towne strain of CMV, a preferred strain for use in preparation of a vaccine of

this invention because of its broad antigenic spectrum and its attenuation, was isolated from the urine of a two month old male infant with cytomegalic inclusion disease (symptoms - central nervous system damage and hepatosplenomegaly). This strain of CMV was isolated by Stanley A. Plotkin, M.D. and is described in J. Virol., 11 (6): 991 (1973). This strain is freely available from The Wistar Institute or from the ATCC under accession number VR-977. However, other strains of CMV useful in the practice of this invention may be obtained from depositories like the ATCC or from other institutes or universities.

In the practice of one embodiment of this invention the HCMV subunit may be produced *in vitro* by recombinant techniques in large quantities sufficient for use in an immunogenic composition or subunit vaccine. Alternatively, the recombinant adenovirus containing the subunit may itself be employed as an immunogenic or vaccine component, capable of expressing the subunit *in vivo*.

The presently preferred subunit proteins for use in the present invention are the HCMV gB subunit fragments. One embodiment of the present invention provides a replication competent (non-defective) adenovirus vector carrying a fragment of the HCMV gB gene which contains a CTL epitope and/or B cell epitope. A preferred gene fragment encodes about amino acid 1 to about amino acid 303 of the gB subunit protein SEQ ID NO:2. Another suitable fragment of gB SEQ ID NO:2 is the fragment spanning about amino acid 1 to about amino acid 700 of SEQ ID NO:2. Still another suitable gB fragment spans about amino acid 1 to about amino acid 465 of SEQ ID NO:2.

More particularly, it is anticipated that smaller fragments containing all or a portion of the gB

fragment spanning amino acids about 155 to about 303 will also be desirable for vaccine use. This region is suspected of containing at least a CTL epitope (see Examples 5 and 6 below).

It is anticipated that in the construction of the adenovirus vectors of this invention, any of the subunits of the HCMV envelope protein may be employed. In a manner similar to the use of the gB fragment in this vaccine, other subunits of CMV which may be employed in the production of a vaccine according to the invention may be selected from the gcII, gcIII, or immediate early subunits of the human virus. Alternatively, more than one HCMV subunit may be employed in a vaccine according to the teachings of the present invention.

In addition to isolating the desired subunit from an available strain of HCMV for insertion into the selected adenovirus, the sequences of the subunits of two HCMV strains have been published [See, e.g., Mach et al, J. Gen. Virol., 67:1461-1467 (1986); Cranage et al, (1986) cited above; and Spaete et al, Virol., 167:207-225 (1987). These subunit sequences can therefore be chemically synthesized by conventional methods known to one of skill in the art, or the sequences purchased from commercial sources.

The recombinant adenovirus of the present invention may also contain multiple copies of the HCMV subunit. Alternatively, the recombinant virus may contain more than one HCMV subunit type, so that the virus may express two or more HCMV subunits, subunit fragments, or immediate early antigens and subunits together.

In the construction of the adenovirus vector of the present invention, the CMV subunit sequence is preferably inserted in an adenovirus strain under the control of an expression control sequence in the virus

itself. The adenovirus vector of the present invention preferably contains other sequences of interest in addition to the HCMV subunit. Such sequences may include regulatory sequences, enhancers, suitable promoters, secretory signal sequences and the like. The techniques employed to insert the subunit sequence into the adenovirus vector and make other alterations in the viral DNA, e.g., to insert linker sequences and the like, are known to one of skill in the art. See, e.g., T. Maniatis et al, "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982). Thus, given the disclosures contained herein the construction of suitable adenovirus expression vectors for expression of an HCMV subunit protein is within the skill of the art. Example 3 below provides construction details for the non-defective adenovirus containing these gB fragments.

The recombinant adenovirus itself, constructed as described above, may be used directly as an immunogen or a vaccine component. According to this embodiment of the invention, the recombinant adenovirus, containing the HCMV subunit, e.g., the gB subunit fragment, is introduced directly into the patient by vaccination. The recombinant virus, when introduced into a patient directly, infects the patient's cells and produces the CMV subunit in the patient's cells. The inventors have found that this method of presenting these HCMV genes to a vaccinate is particularly capable of eliciting an immune response. Examples 5 and 6 demonstrate the ability of a recombinant adenovirus containing the gB fragment, amino acid 1-303 of SEQ ID NO:2, to induce a gB-specific, protective CTL response in mice.

The use of these adenovirus recombinants as immunogens capable of inducing a CTL response is surprising in view of the results obtained in the same

assays of the examples with other known virus types, which have been used in vaccines previously. According to another embodiment of this invention, once the recombinant viral vector containing the CMV subunit protein, e.g., the gB_{1-303} subunit fragment, is constructed, it may be infected into a suitable host cell for *in vitro* expression. The infection of the recombinant viral vector is performed in a conventional manner. [See, Maniatis et al, supra.] Suitable host cells include, without limitation, mammalian cells and cell lines, e.g., A549 (human lung carcinoma) or 293 (transformed human embryonic kidney) cells.

The host cell, once infected with the recombinant virus of the present invention, is then cultured in a suitable medium, such as Minimal Essential Medium (MEM) for mammalian cells. The culture conditions are conventional for the host cell and allow the subunit, e.g., gB_{1-303} subunit fragment, to be produced either intracellularly, or secreted extracellularly into the medium. Conventional protein isolation techniques are employed to isolate the expressed subunit from the selected host cell or medium.

When expressed *in vitro* and isolated from culture, the subunit, e.g., gB_{1-303}, may then be formulated into an appropriate vaccine composition. Such compositions may generally contain one or more of the recombinant CMV subunits.

The preparation of a pharmaceutically acceptable vaccine composition, having appropriate pH, isotonicity, stability and other conventional characteristics is within the skill of the art. Thus such vaccines may optionally contain other components, such as adjuvants and/or carriers, e.g., aqueous suspensions of aluminum and magnesium hydroxides.

Thus, the present invention also includes a method of vaccinating humans against human CMV infection with the recombinant adenovirus vaccine composition. This vaccine composition is preferably orally administered, because adenoviruses are known to replicate in cells of the stomach. Previous studies with adenoviruses have shown them to be safe when administered orally [see, e.g., Collis et al, cited above]. However, the present invention is not limited by the route of administration selected for the vaccine.

When the recombinant adenovirus is administered as the vaccine, a dosage of between 10^5 and 10^8 plaque forming units may be used. Additional doses of the vaccines of this invention may also be administered where considered desirable by the physician. The dosage regimen involved in the method for vaccination against CMV infection with the recombinant virus of the present invention can be determined considering various clinical and environmental factors known to affect vaccine administration.

Alternatively, the vaccine composition may comprise one or more recombinantly-produced human CMV subunit proteins, preferably a fragment of a gB subunit. The *in vitro* produced subunit proteins may be introduced into the patient in a vaccine composition as described above, preferably employing the oral, nasal or subcutaneous routes of administration. The presence of the subunit produced either *in vivo* or as part of an *in vitro* expressed subunit administered with a carrier, stimulates an immune response in the patient. Such an immune response is capable of providing protection against exposure to the whole human CMV microorganism. The dosage for all routes of administration of the *in vitro* vaccine containing one or more of the CMV subunit proteins is generally greater than 20 micrograms of

protein per kg of patient body weight, and preferably between 40 and 80 micrograms of protein per kilogram.

The utility of the recombinant adenoviruses of the present invention is demonstrated through the use of a novel mouse experimental model which characterizes cytotoxic T lymphocyte (CTL) responses to individual proteins of strictly human-restricted viruses. For example, the model as used herein is based on the use of two types of recombinant viruses, an adenovirus and a canarypox virus, both expressing a gene of the same HCMV protein. This model is useful in identifying immunodominant HCMV proteins and immunodominant epitopes of individual proteins to incorporate into an appropriate immunizing vector, analysis of proteins of various HCMV strains, immunization protocols and the longevity of cell-mediated immunity to individual proteins or epitopes; and investigation of the optimal vector for effective introduction of a certain antigen or epitope to the host immune system.

According to this model, mice are immunized with one recombinant of the invention, and CTL activity is tested in target cells infected with the other recombinant. Specifically, Examples 4-6 below provide a murine model of the cytotoxic T lymphocyte (CTL) response to the amino acid 1-303 fragment of the glycoprotein B (gB) gene [SEQ ID NO:2] of human cytomegalovirus (HCMV) based on the use of gB-expressing adenovirus (Ad-gB) and several poxvirus recombinants. Using this model, it has been demonstrated that the human CMV subunit gB (HCMV-gB) amino acid 1-303 fragment can elicit a major histocompatibility complex (MHC) class I-restricted HCMV-gB-specific CTL response in mice.

The following examples illustrate the construction of a non-defective adenovirus strain capable of expressing the HCMV major envelope glycoprotein gB_{1-303}

fragment and the efficacy of these compositions as an HCMV vaccine. These examples are illustrative only and do not limit the scope of the present invention.

Example 1 - Construction of a Non-defective Adenovirus - gB (Ad-gB) Recombinant

The gB gene was cloned from the Towne strain of HCMV [Wistar Institute] as follows. The gB gene was first mapped to the 20.5 kb Hind III D fragment of HCMV using oligonucleotides that corresponded to the 5' and 3' termini of the published AD-169 gB sequence [See, Cranage et al (1986), cited above]. The Hind III fragment was cut with XbaI to generate a 9.8 kb fragment. This fragment was then cut with XmaIII to generate a 3.1 kb fragment. The 3.1 kb XmaIII fragment which contained the gB gene, had XbaI linkers attached to its 5' and 3' termini.

An adenovirus type 5 plasmid, pAd5 Bam-B, which contains the 59.5 - 100 mu region of the Ad5 adenovirus genome cloned into the BamHI site of pBR322 [See, R. L. Berkner et al, Nucl. Acids Res., 11:6003-6020 (1983) and M. E. Morin et al, cited above] was digested with XbaI to remove the 78.5 mu - 84.7 mu sequences of the Ad5 genome. The 78.5 to 84.7 mu deletion removes most of the coding region of the E3 transcription unit of Ad5 but leaves the E3 promoter intact. The XbaI-linked 3.1 kb fragment of CMV containing the gB gene was inserted into this XbaI site of pAd5 Bam-B. Fig. 1A provides a diagrammatic illustration of the above description.

To generate recombinant virus, the 0-76 mu fragment of wild type Ad5 virus was isolated by digesting the viral DNA with EcoRI [See, U. Petterson et al, J. Mol. Biol., 73:125-130 (1973)]. This fragment was co-transfected with the 59.5 to 100 mu BamHI fragment of pAd5 Bam-B containing the gB gene as described above into

human embryonic kidney 293 cells, available from the American Type Culture Collection. The Ad-gB recombinant was generated by overlap recombination between the viral sequences as illustrated in Fig. 1B.

The gB recombinant virus was plaque purified on human lung carcinoma A549 cells [ATCC CCL185] using standard procedures. Viruses containing both orientations of the gB gene, as determined by Southern blotting, were isolated.

The recombinant containing the gB gene in the same 5' to 3' direction as the adenovirus E3 promoter of the adenovirus type 5 strain is under the transcriptional control of the E3 promoter. The plaque purified recombinant virus retains the cloning XbaI sites. The above-described cloned gB gene is devoid of its natural promoter according to the DNA sequence of gB identified in Spaete et al, (1987), cited above.

Example 2 - Production of the Full-Length gB Subunit

The adenovirus gB plasmid construct and the Ad5 mu 0-76 DNA of Example 1 were cotransfected into 293 cells, human cells transformed by adenovirus 5 early genes [See, Graham et al, J. Gen. Virol.,36:59-72 (1977); and ATCC CRL1573] employing conventional procedures. This transfection generated a functional recombinant virus by homologous overlap recombination as shown in Fig. 1B.

Southern blot analysis confirmed the presence of an adenovirus, type 5, containing the HCMV gB subunit (referred to as either Ad-5/gB or Ad-gB) recombinant virus which was subsequently purified by plaque purification using standard procedures.

The recombinant virus AD-5/gB, expresses gB subunit protein as determined by conventional assays, i.e., immunofluorescence on fixed cells and by Western

blot using monospecific guinea pig antiserum and monoclonal antibodies to gB protein [See, e.g., T. Maniatis et al, cited above]. The Ad-5/gB recombinant, also referred to as Ad-gB, is also described in applicant's publication [Marshall et al., J. Infect. Dis., 162:1177-1181 (1990)] published after the filing date of the original parent application from which this application claims priority.

Example 3 - Construction of the gB gene fragments

Ad-gB_{1-303} and Ad-gB_{1-155} recombinant viruses were constructed by overlap recombination as described for Ad-gB in Example 2 above. Briefly, in order to clone the subfragments of the gB gene, five oligonucleotide primers for polymerase chain reactions (PCR) were synthesized. The primers were designed to anneal with various portions of the gB DNA sequence and promote amplification of the gene. In addition, all of the oligonucleotide primers were engineered to contain an Xba I site so that the PCR product could be digested with this enzyme in order to facilitate cloning into the pAd-5 vector.

5' gB primer : SEQ ID NO:3:

4889: 5'-ACACGCAAGAGA TCTAGA CGCGCCTCAT

3' primer at amino acid 700 of gB protein: SEQ ID NO:4:

5'-TCGTCCAGAC TCTAGA GGTAGGGC

3' primer at aa 465: SEQ ID NO:5:

5'-CGACTCCAT TCTAGA TTAATGAGTTGCATT

3' primer at aa 303: SEQ ID NO:6:

5'-CAAAGTCGGAG TCTAGAG TCTAGTTCGGAAA

3' primer at aa 155: SEQ ID NO:7:

5'-CAGATAAGTGG TCTAGA TCTAAGCGTAGCTACG

The above oligonucleotides correspond to the following nucleotide positions of the HCMV gB gene (Towne strain) as reported by Spaete et al, Virology, 167:207-225 (1988). SEQ ID NO:3 corresponds to nucleotide positions

895 to 922 in the sense orientation; SEQ ID NO:4 to nucleotide positions 3090 to 3067 anti-sense; SEQ ID NO:5 to nucleotide positions 2375 to 2350 anti-sense; SEQ ID NO:6 to nucleotide positions 1877 to 1847 anti-sense; and SEQ ID NO:7 to nucleotide positions 1432 to 1400 anti-sense. These immediately preceding nucleotide numbers are not identical to those of SEQ ID NO: 1 because the Spaete et al sequence, to which these numbers correspond, contains additional 5' non-coding sequence while SEQ ID NO: 1 reports only the DNA sequence corresponding to the coding region of the gB protein [SEQ ID NO: 2].

The specific segments or fragments of the gB gene were amplified using the Perkin-Elmer Amplitaq™ kit by mixing 400 ng of the 5' gB primer with each of the 3' primers separately (400 ng of each) and 0.1 μg of purified HCMV genomic DNA or 0.1 μg of previously cloned intact gB gene (see Example 2). The final reaction mixture was 100 μL and the thermocycling conditions were 94°C, 1 minute; 52°C, 1 minute; 72°C, 1 minute, repeated for a total of 35 cycles. Amplified DNA was purified by cutting the proper DNA fragment out of a 1.2% agarose gel, digested with XbaI, repurified by cutting the digested fragments out of a 1.2% agarose gel and then ligated into the XbaI site of the cloning vector pAd-5. Positive recombinants were verified by DNA sequence analysis and sequence analysis confirmed the orientation of the clones.

Example 4 - CTL Assays

A. Recombinant Viruses Used

The following recombinant viruses were used in the CTL assays of Examples 5-6 below to demonstrate the immunogenicity and vaccine utility of the recombinant adenoviruses of the present invention.

Wild-type human adenovirus type 5 (WT-Ad) and the Ad-gB recombinant were propagated in human lung carcinoma A549 cells [ATCC CCL185], as described in Example 1.

An E3-deleted adenovirus type 5 mutant lacking the XbaI D fragment of adenovirus DNA (Ad5ΔE3) was constructed by overlap recombination, using plasmid pAd-5 mu 59.5-100, which was deleted in E3 sequences (mu 78.5-84) using the techniques described in Example 1, and pAd-5 mu 0-75.9 [G. S. Marshall et al, J. Infect. Dis., 162:1177-1181 (1990), hereby incorporated by reference].

A vaccinia virus recombinant containing the gB subunits (VacC-gB) described previously in Gonczol et al, Vaccine, 9:631-637 (1991) and the parental Copenhagen strain of vaccinia, VC-2 (also known as wild-type vaccinia (WT-Vac)) were grown in Vero cells [E. Gonczol et al, Vaccine, 8:130-136 (1990); J. Tartaglia et al, Crit. Rev. Immunol., 10:13-30 (1990)].

The vaccinia WR strain [obtained from Dr. Enzo Paoletti, Virogenetics Corp, Troy, NY] was used to develop a recombinant expressing HCMV-gB ((VacW)-gB). This recombinant was derived using a strategy similar to that described for the VacC-gB recombinant (Gonczol et al., cited above).

A canarypox recombinant [ALVAC-CMV (vCP139) which is subsequently referred to as Cp-gB] expressing the HCMV-gB gene was constructed using a strategy similar to that described for a canarypox-rabies recombinant in Taylor et al., Vaccine, 9:190-193 (1991) [also obtained from Dr. Enzo Paoletti]. Briefly, the gene encoding the HCMV (Towne strain) glycoprotein B was inserted into a canarypox donor plasmid consisting of a polylinker flanked by genomic sequence from which a nonessential gene was specifically deleted (at a unique EcoRI site within a 3.3 kbp PvuII subgenomic fragment of canarypox

DNA). Expression of the gB protein gene was placed under the transcriptional control of an early/late vaccinia virus promoter (H6) previously described [Percus et al., J. Virol., 63:3829-3835 (1989)]. Cp-gB was derived and plaque-purified by standard methods [Panicali and Paoletti, Proc. Natl. Acad. Sci. USA, 79:4927-4931 (1982)]. The Cp-gB recombinant and parental canarypox virus (WT-Cp) were propagated on primary chick embryo fibroblasts.

B. Expression of the gB protein in Cp-gB recombinant virus

Chicken embryo fibroblast (CEF) cells [ATCC CRL 1590] infected with either Cp-gB or with the parental wild-type canarypox (WT-Cp) virus preparations were analyzed by Western blot assay using the 4A guinea-pig serum directed against the gB protein. Western blot assays and the 4A guinea-pig serum, used as gB-specific antibody, were described previously in Gonczol et al., J. Virol., 58:661-664 (1986). Uninfected and HCMV-infected MRC-5 cell [ATCC CCL 171] lysates were included as controls.

A diffuse band at the 140 kDa position and a double band of 55 and 58 kDa were detected in both Cp-gB-infected CEF cells and in HCMV-infected MRC-5 cells. The presence of these gB-specific proteins presumably representing the glycosylated 140 kDa precursor and the differentially glycosylated cleavage products (55 and 58 kDa) indicates that the Cp-gB recombinant expresses the inserted gB gene. The slight difference between the mobility of 55 and 58 kDa cleavage products of control and recombinant gB may reflect different glycosylation patterns.

C. Murine Model and CTL Assay

For immunization of mice, Ad-gB and WT-Ad were purified by CsCl gradient centrifugation. VacC-gB,

VacW-gB and WT-Vac were purified by sucrose gradient centrifugation, and Cp-gB and WT-Cp were concentrated on sucrose cushion.

Six- to 8-week-old female BALB/c and CBA mice (from Harlan Sprague-Dawley and Jackson) and 12-week-old male BALB/k mice (from The Wistar Institute Animal Facility) were immunized intraperitoneally (i.p.) with the recombinant viruses described above at 1-5 x 10^8 pfu unless otherwise stated.

One to 12 weeks later, spleens were aseptically removed and cell suspensions were prepared by gently pressing the spleens through a stainless steel mesh. Cells were suspended at 2.5 x 10^6 viable cells/ml in RPMI 1640 medium containing 5% FBS (Gibco), 2 x 10^{-5} M 2-mercaptoethanol, 14 mM HEPES buffer, glutamine and 50 μg/ml gentamicin. Spleen cell cultures were restimulated *in vitro* with Ad-gB (multiplicity of infection (m.o.i.) = 10) or VacC-gB (m.o.i. = 0.5) infected autologous spleen cells for 5 days in 24-well plates. Cytolytic activity of nonadherent spleen cells was tested in a chromium release assay which was performed as follows.

1. T-cell subset depletion

For *in vitro* depletion of CD4 or CD8 cells, 3 x 10^6 spleen cells were incubated with anti-mouse CD4 monoclonal antibody (MAb) [Pharmingen; Cat.3:01061 D; 20 μg/3x10^6 cells] or CD8 MAb [Accurate; Cat.#:CL-8921; diluted 1:4] for 60 minutes at 4°C, and further incubated in the presence of rabbit complement [Accurate; Low-tox M; diluted 1:10] for 30 minutes at 37°C. The cells were washed twice and used as effector cells in a ^{51}Cr-release test.

2. Chromium release assay

P815 (H-2^d) [ATCC TIB 64], mouse MC57 (H-2^b) cells [also termed MC-57G, D.P. Aden et al, Immunogenetics, 3:209-221 (1976)] and mouse NCTC clone

929 ($H\text{-}2^k$) cells [ATCC CCL 1] were used as target cells. The HCMV neutralization titer of mouse sera was determined on MRC-5 cells [ATCC CCL 171] by the microneutralization method as described in Gonczol et al., J. Virol. Methods, 14:37-41 (1986).

The target cells were infected with Ad-gB or Ad-5ΔE3 (multiplicity of infection (m.o.i.) = 40-80, 40 hours) or with Vac-gB or WT-Vac (m.o.i. = 5-10, 4 hours). Target cells were washed in the modified RPMI 1640 medium described above and 2 x 10^6 cells were labeled with 100 μCi of [^{51}Cr]NaCrO4 [Amersham, specific activity 250-500 mCi/mg] for 1 hour. The labeled target cells were washed 3 times in phosphate-buffered saline (PBS) and then mixed with the effector cells at various effector:target ratios in triplicate using 96-well U-bottomed microtiter plates and incubated for 4 hours.

Percentage specific ^{51}Cr release was calculated as: [(cpm experimental release - cpm spontaneous release) / (cpm maximal release - cpm spontaneous release)] x 100. Standard deviation of the mean of triplicate cultures was less than 10%, and spontaneous release was always less than 25%.

This CTL assay is a system in which two types of viral expression vectors, poxvirus and adenovirus, carrying the same fragment of the HCMV-gB gene, are alternately used for immunization of animal or for infection of target cells to show that HCMV-gB fragment is an inducer of CTL in mice. Using this model system, the relative immunogenicity of the gB fragment expressed by different recombinant viruses has been evaluated.

Example 5 - CTL Responses to Adenovirus Containing gB Fragments

Ad-$gB_{1\text{-}303}$ and Ad-$gB_{1\text{-}155}$ recombinant viruses were constructed as described in Example 3 above.

In CTL experiments performed as described in Example 4, CBA mice were immunized i.p. with 10^8 pfu of the Ad-gB, Ad-gB_{1-303} or Ad-gB_{1-155}. Two weeks later spleen cells were restimulated *in vitro* with Ad-gB infected autologous spleen cells and tested for ability to lyse Wt-Ad, Vac-gB or Wt-Vac infected L929 (MHC-class I matched) cells.

All recombinants showed an Ad virus-specific CTL response, but only Ad-gB (containing the complete gB coding sequence) and Ad-gB_{1-303} exerted gB-specific CTL, indicating the presence of a CTL-epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

Example 6 - Protection Studies with Adenovirus Containing gB Fragments

Using the murine model described in Example 4, CBA mice were immunized with 1 x 10^8 pfu of Wt-Ad, Ad5Δ3 (an E3 deleted mutant virus, the parental strain of the recombinant viruses), Ad-gB, Ad-gB_{1-303} or Ad-gB_{1-155}. Five to ten days later the immunized mice were challenged i.c. with VacWR-gB (a neurovirulent vaccinia strain expressing the HCMV-gB protein). Control mice, immunized with the Wt-Ad or Ad5Δ3 virus died within 4-7 days after the challenge.

Ad-gB and Ad-gB_{1-303}-immunized mice survived (92% and 95% survival, respectively), while all of the Ad-gB_{1-155}-immunized mice died, indicating a protection epitope on the N-terminal part of the gB protein between amino acid 155 and 303.

Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. For example, use of other appropriate non-defective adenovirus strains for construction of

analogous expression systems to express the HCMV gB fragment may be constructed according to the disclosure of the present invention.

Additionally, the other subunits of HCMV major glycoprotein complexes, e.g., gcII or gcIII, or immediate-early antigens, may be expressed in a non-defective adenovirus recombinant in the same manner as described above for subunit gB fragment. Such modifications and alterations to the compositions and processes of the present invention are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Wistar Institute of Anatomy, Biology
Government of USA Dept.
Health and Human Services

(ii) TITLE OF INVENTION: Recombinant Cytomegalovirus Vaccine

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Howson and Howson
(B) STREET: Spring House Corporate Center, PO Box 457
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(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19477

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/048,978
(B) FILING DATE: 16-APR-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bak, Mary E.
(B) REGISTRATION NUMBER: 31,215
(C) REFERENCE/DOCKET NUMBER: WST6CPCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 215-540-9200
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2724 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2721

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATG GAA TCC AGG ATC TGG TGC CTG GTA GTC TGC GTT AAC TTG      42
Met Glu Ser Arg Ile Trp Cys Leu Val Val Cys Val Asn Leu
 1               5                  10

TGT ATC GTC TGT CTG GGT GCT GCG GTT TCC TCA TCT TCT ACT      84
Cys Ile Val Cys Leu Gly Ala Ala Val Ser Ser Ser Ser Thr
 15                  20                  25

CGT GGA ACT TCT GCT ACT CAC AGT CAC CAT TCC TCT CAT ACG     126
Arg Gly Thr Ser Ala Thr His Ser His His Ser Ser His Thr
     30                  35                  40

ACG TCT GCT GCT CAT TCT CGA TCC GGT TCA GTC TCT CAA CGC     168
Thr Ser Ala Ala His Ser Arg Ser Gly Ser Val Ser Gln Arg
         45                  50                  55

GTA ACT TCT TCC CAA ACG GTC AGC CAT GGT GTT AAC GAG ACC     210
Val Thr Ser Ser Gln Thr Val Ser His Gly Val Asn Glu Thr
             60                  65                  70

ATC TAC AAC ACT ACC CTC AAG TAC GGA GAT GTG GTG GGG GTC     252
Ile Tyr Asn Thr Thr Leu Lys Tyr Gly Asp Val Val Gly Val
                 75                  80

AAC ACC ACC AAG TAC CCC TAT CGC GTG TGT TCT ATG GCA CAG     294
Asn Thr Thr Lys Tyr Pro Tyr Arg Val Cys Ser Met Ala Gln
 85                  90                  95

GGT ACG GAT CTT ATT CGC TTT GAA CGT AAT ATC GTC TGC ACC     336
Gly Thr Asp Leu Ile Arg Phe Glu Arg Asn Ile Val Cys Thr
    100                 105                 110

TCG ATG AAG CCC ATC AAT GAA GAC CTG GAC GAG GGC ATC ATG     378
Ser Met Lys Pro Ile Asn Glu Asp Leu Asp Glu Gly Ile Met
        115                 120                 125
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GTG GTC TAC AAA CGC AAC ATC GTC GCG CAC ACC TTT AAG GTA      420
Val Val Tyr Lys Arg Asn Ile Val Ala His Thr Phe Lys Val
            130                 135                 140

CGA GTC TAC CAG AAG GTT TTG ACG TTT CGT CGT AGC TAC GCT      462
Arg Val Tyr Gln Lys Val Leu Thr Phe Arg Arg Ser Tyr Ala
                145                 150

TAC ATC CAC ACC ACT TAT CTG CTG GGC AGC AAC ACG GAA TAC      504
Tyr Ile His Thr Thr Tyr Leu Leu Gly Ser Asn Thr Glu Tyr
155                 160                 165

GTG GCG CCT CCT ATG TGG GAG ATT CAT CAT ATC AAC AGT CAC      546
Val Ala Pro Pro Met Trp Glu Ile His His Ile Asn Ser His
    170                 175                 180

AGT CAG TGC TAC AGT TCC TAC AGC CGC GTT ATA GCA GGC ACG      588
Ser Gln Cys Tyr Ser Ser Tyr Ser Arg Val Ile Ala Gly Thr
        185                 190                 195

GTT TTC GTG GCT TAT CAT AGG GAC AGC TAT GAA AAC AAA ACC      630
Val Phe Val Ala Tyr His Arg Asp Ser Tyr Glu Asn Lys Thr
            200                 205                 210

ATG CAA TTA ATG CCC GAC GAT TAT TCC AAC ACC CAC AGT ACC      672
Met Gln Leu Met Pro Asp Asp Tyr Ser Asn Thr His Ser Thr
                215                 220

CGT TAC GTG ACG GTC AAG GAT CAA TGG CAC AGC CGC GGC AGC      714
Arg Tyr Val Thr Val Lys Asp Gln Trp His Ser Arg Gly Ser
225                 230                 235

ACC TGG CTC TAT CGT GAG ACC TGT AAT CTG AAT TGT ATG GTG      756
Thr Trp Leu Tyr Arg Glu Thr Cys Asn Leu Asn Cys Met Val
    240                 245                 250

ACC ATC ACT ACT GCG CGC TCC AAG TAT CCC TAT CAT TTT TTC      798
Thr Ile Thr Thr Ala Arg Ser Lys Tyr Pro Tyr His Phe Phe
        255                 260                 265

GCA ACT TCC ACG GGT GAT GTG GTT GAC ATT TCT CCT TTC TAC      840
Ala Thr Ser Thr Gly Asp Val Val Asp Ile Ser Pro Phe Tyr
            270                 275                 280

AAC GGA ACT AAT CGC AAT GCC AGC TAT TTT GGA GAA AAC GCC      882
Asn Gly Thr Asn Arg Asn Ala Ser Tyr Phe Gly Glu Asn Ala
                285                 290

GAC AAG TTT TTC ATT TTT CCG AAC TAC ACT ATC GTC TCC GAC      924
Asp Lys Phe Phe Ile Phe Pro Asn Tyr Thr Ile Val Ser Asp
295                 300                 305
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TTT GGA AGA CCG AAT TCT GCG TTA GAG ACC CAC AGG TTG GTG     966
Phe Gly Arg Pro Asn Ser Ala Leu Glu Thr His Arg Leu Val
    310                 315                 320

GCT TTT CTT GAA CGT GCG GAC TCA GTG ATC TCC TGG GAT ATA    1008
Ala Phe Leu Glu Arg Ala Asp Ser Val Ile Ser Trp Asp Ile
        325                 330                 335

CAG GAC GAG AAG AAT GTT ACT TGT CAA CTC ACT TTC TGG GAA    1050
Gln Asp Glu Lys Asn Val Thr Cys Gln Leu Thr Phe Trp Glu
            340                 345                 350

GCC TCG GAA CGC ACC ATT CGT TCC GAA GCC GAG GAC TCG TAT    1092
Ala Ser Glu Arg Thr Ile Arg Ser Glu Ala Glu Asp Ser Tyr
                355                 360

CAC TTT TCT TCT GCC AAA ATG ACC GCC ACT TTC TTA TCT AAG    1134
His Phe Ser Ser Ala Lys Met Thr Ala Thr Phe Leu Ser Lys
365                 370                 375

AAG CAA GAG GTG AAC ATG TCC GAC TCT GCG CTG GAC TGT GTA    1176
Lys Gln Glu Val Asn Met Ser Asp Ser Ala Leu Asp Cys Val
    380                 385                 390

CGT GAT GAG GCC ATA AAT AAG TTA CAG CAG ATT TTC AAT ACT    1218
Arg Asp Glu Ala Ile Asn Lys Leu Gln Gln Ile Phe Asn Thr
        395                 400                 405

TCA TAC AAT CAA ACA TAT GAA AAA TAT GGA AAC GTG TCC GTC    1260
Ser Tyr Asn Gln Thr Tyr Glu Lys Tyr Gly Asn Val Ser Val
            410                 415                 420

TTT GAA ACC ACT GGT GGT TTG GTG GTG TTC TGG CAA GGT ATC    1302
Phe Glu Thr Thr Gly Gly Leu Val Val Phe Trp Gln Gly Ile
                425                 430

AAG CAA AAA TCT CTG GTG GAA CTC GAA CGT TTG GCC AAC CGC    1344
Lys Gln Lys Ser Leu Val Glu Leu Glu Arg Leu Ala Asn Arg
435                 440                 445

TCC AGT CTG AAT CTT ACT CAT AAT AGA ACC AAA AGA AGT ACA    1386
Ser Ser Leu Asn Leu Thr His Asn Arg Thr Lys Arg Ser Thr
    450                 455                 460

GAT GGC AAC AAT GCA ACT CAT TTA TCC AAC ATG GAG TCG GTG    1428
Asp Gly Asn Asn Ala Thr His Leu Ser Asn Met Glu Ser Val
        465                 470                 475

CAC AAT CTG GTC TAC GCC CAG CTG CAG TTC ACC TAT GAC ACG    1470
His Asn Leu Val Tyr Ala Gln Leu Gln Phe Thr Tyr Asp Thr
            480                 485                 490
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TTG CGC GGT TAC ATC AAC CGG GCG CTG GCG CAA ATC GCA GAA   1512
Leu Arg Gly Tyr Ile Asn Arg Ala Leu Ala Gln Ile Ala Glu
                495                 500

GCC TGG TGT GTG GAT CAA CGG CGC ACC CTA GAG GTC TTC AAG   1554
Ala Trp Cys Val Asp Gln Arg Arg Thr Leu Glu Val Phe Lys
505                 510                 515

GAA CTT AGC AAG ATC AAC CCG TCA GCT ATT CTC TCG GCC ATC   1596
Glu Leu Ser Lys Ile Asn Pro Ser Ala Ile Leu Ser Ala Ile
    520                 525                 530

TAC AAC AAA CCG ATT GCC GCG CGT TTC ATG GGT GAT GTC CTG   1638
Tyr Asn Lys Pro Ile Ala Ala Arg Phe Met Gly Asp Val Leu
        535                 540                 545

GGT CTG GCC AGC TGC GTG ACC ATT AAC CAA ACC AGC GTC AAG   1680
Gly Leu Ala Ser Cys Val Thr Ile Asn Gln Thr Ser Val Lys
            550                 555                 560

GTG CTG CGT GAT ATG AAT GTG AAG GAA TCG CCA GGA CGC TGC   1722
Val Leu Arg Asp Met Asn Val Lys Glu Ser Pro Gly Arg Cys
                565                 570

TAC TCA CGA CCA GTG GTC ATC TTT AAT TTC GCC AAC AGC TCG   1764
Tyr Ser Arg Pro Val Val Ile Phe Asn Phe Ala Asn Ser Ser
575                 580                 585

TAC GTG CAG TAC GGT CAA CTG GGC GAG GAT AAC GAA ATC CTG   1806
Tyr Val Gln Tyr Gly Gln Leu Gly Glu Asp Asn Glu Ile Leu
    590                 595                 600

TTG GGC AAC CAC CGC ACT GAG GAA TGT CAG CTT CCC AGC CTC   1848
Leu Gly Asn His Arg Thr Glu Glu Cys Gln Leu Pro Ser Leu
        605                 610                 615

AAG ATC TTC ATC GCC GGC AAC TCG GCC TAC GAG TAC GTG GAC   1890
Lys Ile Phe Ile Ala Gly Asn Ser Ala Tyr Glu Tyr Val Asp
            620                 625                 630

TAC CTC TTC AAA CGC ATG ATT GAC CTC AGC AGC ATC TCC ACC   1932
Tyr Leu Phe Lys Arg Met Ile Asp Leu Ser Ser Ile Ser Thr
                635                 640

GTC GAC AGC ATG ATC GCC CTA GAC ATC GAC CCG CTG GAA AAC   1974
Val Asp Ser Met Ile Ala Leu Asp Ile Asp Pro Leu Glu Asn
645                 650                 655

ACC GAC TTC AGG GTA CTG GAA CTT TAC TCG CAG AAA GAA TTG   2016
Thr Asp Phe Arg Val Leu Glu Leu Tyr Ser Gln Lys Glu Leu
    660                 665                 670
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CGT TCC AGC AAC GTT TTT GAT CTC GAG GAG ATC ATG CGC GAG   2058
Arg Ser Ser Asn Val Phe Asp Leu Glu Glu Ile Met Arg Glu
        675                 680                 685

TTC AAT TCG TAT AAG CAG CGG GTA AAG TAC GTG GAG GAC AAG   2100
Phe Asn Ser Tyr Lys Gln Arg Val Lys Tyr Val Glu Asp Lys
            690                 695                 700

GTA GTC GAC CCG CTG CCG CCC TAC CTC AAG GGT CTG GAC GAC   2142
Val Val Asp Pro Leu Pro Pro Tyr Leu Lys Gly Leu Asp Asp
                705                 710

CTC ATG AGC GGC CTG GGC GCC GCG GGA AAG GCC GTT GGC GTA   2184
Leu Met Ser Gly Leu Gly Ala Ala Gly Lys Ala Val Gly Val
715                 720                 725

GCC ATT GGG GCC GTG GGT GGC GCG GTG GCC TCC GTG GTC GAA   2226
Ala Ile Gly Ala Val Gly Gly Ala Val Ala Ser Val Val Glu
    730                 735                 740

GGC GTT GCC ACC TTC CTC AAA AAC CCC TTC GGA GCC TTC ACC   2268
Gly Val Ala Thr Phe Leu Lys Asn Pro Phe Gly Ala Phe Thr
        745                 750                 755

ATC ATC CTC GTG GCC ATA GCC GTC GTC ATT ATC ATT TAT TTG   2310
Ile Ile Leu Val Ala Ile Ala Val Val Ile Ile Ile Tyr Leu
            760                 765                 770

ATC TAT ACT CGA CAG CGG CGT CTC TGC ATG CAG CCG CTG CAG   2352
Ile Tyr Thr Arg Gln Arg Arg Leu Cys Met Gln Pro Leu Gln
                775                 780

AAC CTC TTT CCC TAT CTG GTG TCC GCC GAC GGG ACC ACC GTG   2394
Asn Leu Phe Pro Tyr Leu Val Ser Ala Asp Gly Thr Thr Val
785                 790                 795

ACG TCG GGC AAC ACC AAA GAC ACG TCG TTA CAG GCT CCG CCT   2436
Thr Ser Gly Asn Thr Lys Asp Thr Ser Leu Gln Ala Pro Pro
    800                 805                 810

TCC TAC GAG GAA AGT GTT TAT AAT TCT GGT CGC AAA GGA CCG   2478
Ser Tyr Glu Glu Ser Val Tyr Asn Ser Gly Arg Lys Gly Pro
        815                 820                 825

GGA CCA CCG TCG TCT GAT GCA TCC ACG GCG GCT CCG CCT TAC   2520
Gly Pro Pro Ser Ser Asp Ala Ser Thr Ala Ala Pro Pro Tyr
            830                 835                 840

ACC AAC GAG CAG GCT TAC CAG ATG CTT CTG GCC CTG GTC CGT   2562
Thr Asn Glu Gln Ala Tyr Gln Met Leu Leu Ala Leu Val Arg
                845                 850
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CTG GAC GCA GAG CAG CGA GCG CAG CAG AAC GGT ACA GAT TCT   2604
Leu Asp Ala Glu Gln Arg Ala Gln Gln Asn Gly Thr Asp Ser
855                 860                 865

TTG GAC GGA CAG ACT GGC ACG CAG GAC AAG GGA CAG AAG CCC   2646
Leu Asp Gly Gln Thr Gly Thr Gln Asp Lys Gly Gln Lys Pro
    870                 875                 880

AAC CTG CTA GAC CGA CTG CGA CAC CGC AAA AAC GGC TAC CGA   2688
Asn Leu Leu Asp Arg Leu Arg His Arg Lys Asn Gly Tyr Arg
        885                 890                 895

CAC TTG AAA GAC TCC GAC GAA GAA GAG AAC GTC TGA           2724
His Leu Lys Asp Ser Asp Glu Glu Glu Asn Val
            900                 905
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 907 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Glu Ser Arg Ile Trp Cys Leu Val Val Cys Val Asn Leu Cys Ile
 1               5                  10                  15

Val Cys Leu Gly Ala Ala Val Ser Ser Ser Ser Thr Arg Gly Thr Ser
            20                  25                  30

Ala Thr His Ser His His Ser Ser His Thr Thr Ser Ala Ala His Ser
        35                  40                  45

Arg Ser Gly Ser Val Ser Gln Arg Val Thr Ser Ser Gln Thr Val Ser
    50                  55                  60

His Gly Val Asn Glu Thr Ile Tyr Asn Thr Thr Leu Lys Tyr Gly Asp
65                  70                  75                  80

Val Val Gly Val Asn Thr Thr Lys Tyr Pro Tyr Arg Val Cys Ser Met
                85                  90                  95

Ala Gln Gly Thr Asp Leu Ile Arg Phe Glu Arg Asn Ile Val Cys Thr
            100                 105                 110

Ser Met Lys Pro Ile Asn Glu Asp Leu Asp Glu Gly Ile Met Val Val
        115                 120                 125
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Tyr Lys Arg Asn Ile Val Ala His Thr Phe Lys Val Arg Val Tyr Gln
    130                 135                 140

Lys Val Leu Thr Phe Arg Arg Ser Tyr Ala Tyr Ile His Thr Thr Tyr
145                 150                 155                 160

Leu Leu Gly Ser Asn Thr Glu Tyr Val Ala Pro Pro Met Trp Glu Ile
                165                 170                 175

His His Ile Asn Ser His Ser Gln Cys Tyr Ser Ser Tyr Ser Arg Val
            180                 185                 190

Ile Ala Gly Thr Val Phe Val Ala Tyr His Arg Asp Ser Tyr Glu Asn
        195                 200                 205

Lys Thr Met Gln Leu Met Pro Asp Asp Tyr Ser Asn Thr His Ser Thr
    210                 215                 220

Arg Tyr Val Thr Val Lys Asp Gln Trp His Ser Arg Gly Ser Thr Trp
225                 230                 235                 240

Leu Tyr Arg Glu Thr Cys Asn Leu Asn Cys Met Val Thr Ile Thr Thr
                245                 250                 255

Ala Arg Ser Lys Tyr Pro Tyr His Phe Phe Ala Thr Ser Thr Gly Asp
            260                 265                 270

Val Val Asp Ile Ser Pro Phe Tyr Asn Gly Thr Asn Arg Asn Ala Ser
        275                 280                 285

Tyr Phe Gly Glu Asn Ala Asp Lys Phe Phe Ile Phe Pro Asn Tyr Thr
    290                 295                 300

Ile Val Ser Asp Phe Gly Arg Pro Asn Ser Ala Leu Glu Thr His Arg
305                 310                 315                 320

Leu Val Ala Phe Leu Glu Arg Ala Asp Ser Val Ile Ser Trp Asp Ile
                325                 330                 335

Gln Asp Glu Lys Asn Val Thr Cys Gln Leu Thr Phe Trp Glu Ala Ser
            340                 345                 350

Glu Arg Thr Ile Arg Ser Glu Ala Glu Asp Ser Tyr His Phe Ser Ser
        355                 360                 365

Ala Lys Met Thr Ala Thr Phe Leu Ser Lys Lys Gln Glu Val Asn Met
    370                 375                 380

Ser Asp Ser Ala Leu Asp Cys Val Arg Asp Glu Ala Ile Asn Lys Leu
385                 390                 395                 400
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Gln Gln Ile Phe Asn Thr Ser Tyr Asn Gln Thr Tyr Glu Lys Tyr Gly
405 410 415

Asn Val Ser Val Phe Glu Thr Thr Gly Gly Leu Val Val Phe Trp Gln
420 425 430

Gly Ile Lys Gln Lys Ser Leu Val Glu Leu Glu Arg Leu Ala Asn Arg
435 440 445

Ser Ser Leu Asn Leu Thr His Asn Arg Thr Lys Arg Ser Thr Asp Gly
450 455 460

Asn Asn Ala Thr His Leu Ser Asn Met Glu Ser Val His Asn Leu Val
465 470 475 480

Tyr Ala Gln Leu Gln Phe Thr Tyr Asp Thr Leu Arg Gly Tyr Ile Asn
485 490 495

Arg Ala Leu Ala Gln Ile Ala Glu Ala Trp Cys Val Asp Gln Arg Arg
500 505 510

Thr Leu Glu Val Phe Lys Glu Leu Ser Lys Ile Asn Pro Ser Ala Ile
515 520 525

Leu Ser Ala Ile Tyr Asn Lys Pro Ile Ala Ala Arg Phe Met Gly Asp
530 535 540

Val Leu Gly Leu Ala Ser Cys Val Thr Ile Asn Gln Thr Ser Val Lys
545 550 555 560

Val Leu Arg Asp Met Asn Val Lys Glu Ser Pro Gly Arg Cys Tyr Ser
565 570 575

Arg Pro Val Val Ile Phe Asn Phe Ala Asn Ser Ser Tyr Val Gln Tyr
580 585 590

Gly Gln Leu Gly Glu Asp Asn Glu Ile Leu Leu Gly Asn His Arg Thr
595 600 605

Glu Glu Cys Gln Leu Pro Ser Leu Lys Ile Phe Ile Ala Gly Asn Ser
610 615 620

Ala Tyr Glu Tyr Val Asp Tyr Leu Phe Lys Arg Met Ile Asp Leu Ser
625 630 635 640

Ser Ile Ser Thr Val Asp Ser Met Ile Ala Leu Asp Ile Asp Pro Leu
645 650 655

Glu Asn Thr Asp Phe Arg Val Leu Glu Leu Tyr Ser Gln Lys Glu Leu
660 665 670

Arg Ser Ser Asn Val Phe Asp Leu Glu Glu Ile Met Arg Glu Phe Asn
675 680 685

Ser Tyr Lys Gln Arg Val Lys Tyr Val Glu Asp Lys Val Val Asp Pro
690 695 700

Leu Pro Pro Tyr Leu Lys Gly Leu Asp Asp Leu Met Ser Gly Leu Gly
705 710 715 720

Ala Ala Gly Lys Ala Val Gly Val Ala Ile Gly Ala Val Gly Gly Ala
725 730 735

Val Ala Ser Val Val Glu Gly Val Ala Thr Phe Leu Lys Asn Pro Phe
740 745 750

Gly Ala Phe Thr Ile Ile Leu Val Ala Ile Ala Val Val Ile Ile Ile
755 760 765

Tyr Leu Ile Tyr Thr Arg Gln Arg Arg Leu Cys Met Gln Pro Leu Gln
770 775 780

Asn Leu Phe Pro Tyr Leu Val Ser Ala Asp Gly Thr Thr Val Thr Ser
785 790 795 800

Gly Asn Thr Lys Asp Thr Ser Leu Gln Ala Pro Pro Ser Tyr Glu Glu
805 810 815

Ser Val Tyr Asn Ser Gly Arg Lys Gly Pro Gly Pro Pro Ser Ser Asp
820 825 830

Ala Ser Thr Ala Ala Pro Pro Tyr Thr Asn Glu Gln Ala Tyr Gln Met
835 840 845

Leu Leu Ala Leu Val Arg Leu Asp Ala Glu Gln Arg Ala Gln Gln Asn
850 855 860

Gly Thr Asp Ser Leu Asp Gly Gln Thr Gly Thr Gln Asp Lys Gly Gln
865 870 875 880

Lys Pro Asn Leu Leu Asp Arg Leu Arg His Arg Lys Asn Gly Tyr Arg
885 890 895

His Leu Lys Asp Ser Asp Glu Glu Glu Asn Val
900 905

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACACGCAAGA GATCTAGACG CGCCTCAT 28

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGTCCAGAC TCTAGAGGTA GGGC 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGACTCCATT CTAGATTAAT GAGTTGCATT 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CAAAGTCGGA GTCTAGAGTC TAGTTCGGAA A 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGATAAGTG GTCTAGATCT AAGCGTAGCT ACG 33

WHAT IS CLAIMED IS:

1. A non-defective recombinant adenovirus containing a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence, said virus capable of expressing said subunit protein.

2. The adenovirus according to claim 1 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

3. An immunogenic composition comprising a a non-defective recombinant adenovirus and a suitable pharmaceutical carrier, wherein said recombinant adenovirus comprises a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus is capable of expressing said subunit protein *in vivo* in an animal.

4. The composition according to claim 3 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

5. The composition according to claim 3 wherein said protein gene encodes an additional cytomegalovirus subunit protein fragment or a selected cytomegalovirus subunit protein.

6. The composition according to claim 3 wherein said gB subunit fragment is about amino acid 1 to about amino acid 303 of SEQ ID NO:2.

7. The composition according to claim 3 wherein said adenovirus is selected from the group consisting of an adenovirus type 5, adenovirus type 4 and adenovirus type 7 strain.

8. The composition according to claim 7 wherein said gB subunit fragment is obtained from the Towne strain cytomegalovirus, and the adenovirus is type 5.

9. The use of a non-defective recombinant adenovirus comprising a human cytomegalovirus protein gene encoding a cytomegalovirus protein gB subunit fragment containing at least one CTL epitope, said gene being under the control of an expression control sequence and said virus being capable of expressing said subunit protein *in vivo* in an animal, in the preparation of a CMV vaccine.

10. The use according to claim 9 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

11. The use according to claim 9 wherein said adenovirus is present in an effective amount of between 10^5 to 10^8 plaque forming units.

12. An immunogenic composition comprising a gB subunit protein fragment containing at least one CTL epitope expressed in a recombinant adenovirus vector.

13. The composition according to claim 12 wherein said fragment is selected from the group consisting of:

(a) the fragment spanning about amino acid 1 to about amino acid 303,

(b) the fragment spanning about amino acid 1 to about amino acid 700,

(c) the fragment spanning about amino acid 1 to about amino acid 465,

(d) fragments spanning about amino acid 155 to about amino acid 303, and

(e) smaller fragments of (a) through (d) of SEQ ID NO:2.

FIG.IA

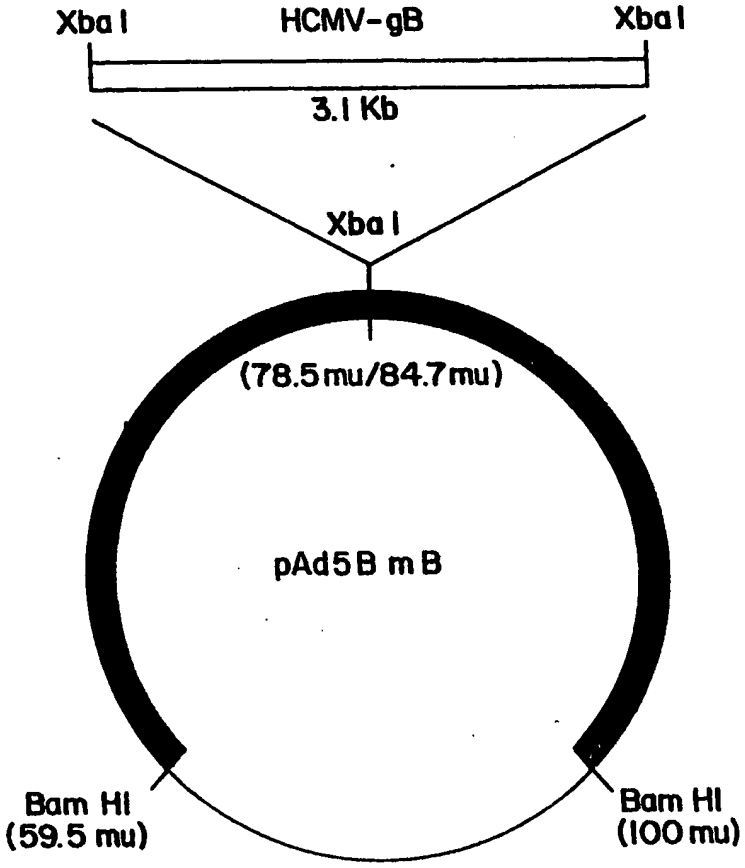
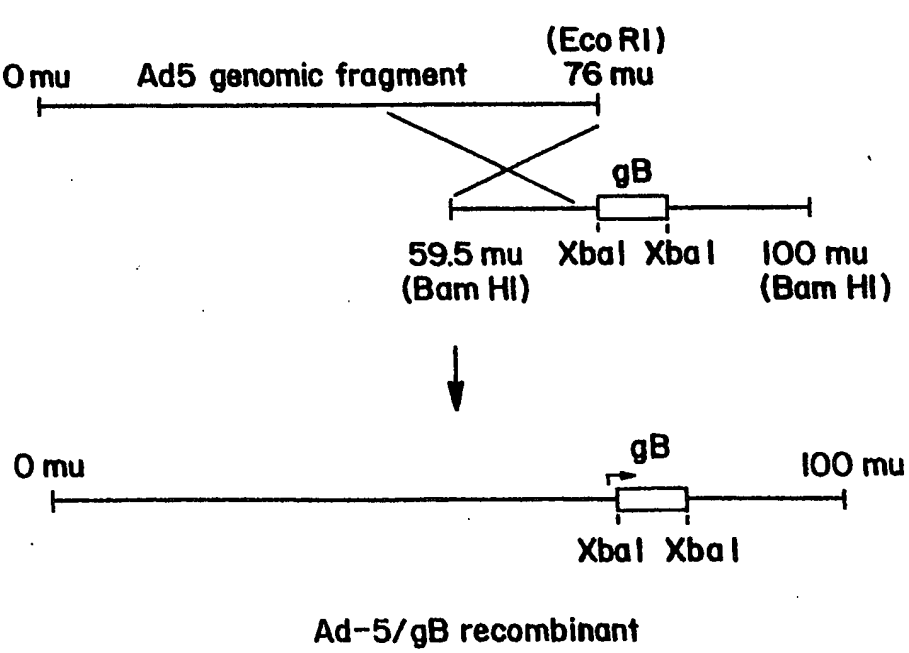

Xba I
HCMV-gB
Xba I
3.1 Kb
Xba I
(78.5mu/84.7mu)
pAd5B mB
Bam HI
(59.5 mu)
Bam HI
(100 mu)

FIG.IB


(Eco RI)
0 mu
Ad5 genomic fragment
76 mu
gB
59.5 mu
(Bam HI)
Xba I
Xba I
100 mu
(Bam HI)
0 mu
gB
100 mu
Xba I
Xba I
Ad-5/gB recombinant

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04180

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 89; 435/235.1, 69.3, 5, 69.1, 172.3; 436/543; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

INTELLIGENETICS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THE EMBO JOURNAL, Volume 5, No. 11, issued November 1986, Cranage et al., "Identification Of The Human Cytomegalovirus Glycoprotein B Gene and Induction of Neutralizing Antibodies via Its Expression in Recombinant Vaccinia Virus", pages 3057-3063, see entire document.	1-13
Y	US, A, 4,920,209 (DAVIS ET AL.) 24 April 1990, see entire document.	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 JULY 1994	02 AUG 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer LAURIE SCHEINER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)

International application No.
PCT/US94/04180

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 39/00, 39/12; C12N 7/00, 15/00; C12P 21/06; C12Q 1/70; G01N 33/531; A01N 43/04